VOLUME LXXII NUMBER 3

THE

BOTANICAL GAZETTE

SEPTEMBER 1921

EFFECT OF DIRECT CURRENT ON CELLS OF ROOT TIP OF CANADA FIELD PEA

HENRY F. A. MEIER

(WITH PLATES II, III, AND THREE FIGURES)

Introduction

EFFECT OF CURRENT ON LIVING STRUCTURES

The numerous researches to determine the effect of the electric current on plants may be divided into two general classes: first, those in which certain organs or entire plants were subjected to electricity, the effect being measured by increase or absence of growth; second, those in which the effect of the current on the individual protoplast formed the basis of study. Among those interested in problems of the second class, AMICI (1) as early as 1818 suggested, although without experimental evidence, that protoplasmic streaming was of electrical origin. Impressed by AMICI'S suggestion, and by the striking results of his own and DUTROCHET'S work on the relation of temperature to protoplasmic streaming, BECQUEREL (2) attempted to show that the direct electric current had the same effect on protoplasmic streaming as variations in temperature. He placed cells of *Chara* in a helix, some parallel and others at right angles to the direction of the electric current, using a battery of 10–30 elements, without in any way influencing the rate of flow. With stronger currents, making direct connection with the cells by means of platinum electrodes,

protoplasmic streaming was inhibited. After a time the flow was resumed. No disorganization of the cells occurred.

JÜRGENSEN (12) worked with *Vallisneria*, noting the effect of the direct current on protoplasmic rotation. He placed sections of leaves on a special object holder, in distilled water, with copper electrodes in contact with the ends of the section. The effect was observed with a microscope giving a magnification of 235–680 diameters. Using 2–4 Grove cells, the speed of rotation of protoplasm was decreased, and long continued exposure to such weak currents brought about an inhibition of the streaming. If the current was discontinued after slowing down the rotation, but before it had entirely stopped, the original speed of rotation was reacquired after a time. When rotation had completely stopped, even though the current was broken immediately, movement was never resumed. Stronger currents produced the same effect as the weaker currents in much less time, the current from 30 cells sufficing to produce immediate and permanent inhibition of rotation. If the current was continued, the protoplasm contracted and gradually migrated toward the end of the cell nearest the anode, where it formed a dense mass against the wall. At break of current this mass would rebound toward the opposite end of the cell. On reversing the direction of current the mass migrated toward the opposite end of the cell, that is, toward the now positive end. JÜRGENSEN regarded these phenomena as coordinate with results he had previously obtained with unorganized bodies.

DU BOIS-REYMOND (7) had previously published similar results, experimenting with starch grains in the cells of a section of living potato tuber. Movement toward the anode was observed, and, as in *Vallisneria* cells, a reversal of current brought the starch grains to the opposite wall.

KÜHNE (14), using the direct current on a plasmodium of a myxomycete, grown on the slide between platinum electrodes 4 mm. apart, reported nuclei moving toward the anode, the cytoplasm toward the cathode. In the cells of *Tradescantia* stamen hairs the entire cell contents moved toward the anode. The ends of the cells toward the cathode changed from their characteristic purple to green, and the opposite end changed to a light red color. KÜHNE

experimented further with the effect of the current on unicellular organisms. Using platinum electrodes, he passed a direct current through a drop of water containing *Actinosphaerium*. The first visible effect of a weak current was the contraction of the pseudopodia lying in the direction of the electrodes. If the current was continued or increased, the pseudopodia lying in the path of the current became vacuolated, the vacuoles on the periphery burst, and the protoplasm of the rounded central portion of the organism began to disintegrate on the side toward the anode. This continued until the whole organism was disintegrated. KÜHNE states that the phenomena described for *Actinosphaerium* hold in general for such forms.

VERWORN (21) repeated and verified KÜHNE'S observations, using non-polarizable electrodes and extending the work considerably, especially with reference to free-swimming protozoa. He found in *Paramoecium* and other free-swimming forms a shrinking at the end toward the anode and a swelling at the opposite end, which phenomenon he regards as illustrating a general tendency to increased contraction on the side toward the anode. He found that some protozoa migrate toward the anode, others toward the cathode.

CARLGREN (3) observed that in *Volvox* the long axis of the colony is placed parallel to the lines of the current, and that there is a movement toward the cathode. If the current is long continued, the colonies move away from the cathode and sometimes gather at the anode. The movement of the flagella on the side toward the anode was inhibited, that on the opposite side not affected. Similarly to VERWORN'S observation on *Paramoecium*, CARLGREN noticed in *Volvox* an anodal shrinking and cathodal swelling with migration of the colony-forming cells (gonidia) within fixed colonies toward the anode. It is interesting to note his further statement that strong currents produced shrinking and swelling on the sides toward the anode and cathode respectively in dead specimens. Furthermore, he produced this shrinking and swelling in dead *Paramoecia* and *Amoebae*. CARLGREN concludes that the physical effect of the current (electrophoresis) accounts for many of the supposed stimulation effects on free swimming forms, and that it plays a large part in electrotaxis.

DALE (6) experimented with the effect of the current on five different species of infusoria found parasitic in the intestine of the frog. The organisms were exposed to the current in various solutions: neutral isotonic saline, slightly acid, and slightly alkaline solutions (using litmus as indicator). The organisms were exposed to the current in a trough with unglazed earthenware sides about 1 cm. apart, mounted on a slide, the ends of the trough being made of sealing wax. Non-polarizable brush electrodes carried the current to the porous earthenware sides. The results were very interesting. In slightly alkaline solution the organisms migrated toward the anode; when in slightly acid solution, toward the cathode. It is true that not all of the five species examined were equally sensitive to the acid and alkali treatment; that is, it required longer treatment in the solutions for some species than for others in order to produce the same effect. In more concentrated salt solutions (good electrical conductors) the migration of the organisms was inhibited.

More recently LILLIE (15) exposed to the direct current various animal structures: isolated nuclei, nuclei of spermatozoa, small leucocytes, and nuclei from lymphoid tissue, also muscle cells teased out in sugar solution, red blood corpuscles, and larger forms of leucocytes. These were suspended in N/4 cane-sugar solution (iso-osmotic with physiological salt solution). In freshly drawn frog's blood, the majority of the red corpuscles moved slowly (at an average speed of 120–130 μ per minute) toward the anode, many showed no migration whatever, and a few moved toward the cathode. The minute lymphocytes moved more rapidly toward the anode at a speed of 1500 μ per minute. The medium sized leucocytes were usually slightly negative (moved toward the anode) or indifferent. The larger leucocytes, however, with more cytoplasm, were in almost all cases decidedly positive (moved toward the cathode). The nuclei obtained by teasing thymus gland and the heads of spermatozoa moved rapidly toward the anode. The rate of the latter was about 2.0 mm. per minute. LILLIE'S conclusion, that "the direction and speed of living cells and portions of tissues are chiefly dependent on the electrical characteristics of their constituent colloids," seems justified.

In 1914 HARDY (9) published a short note on the migration under influence of the direct current of the contents of cells of the onion root tip. His methods and results were briefly as follows. The roots were placed horizontally between non-polarizable electrodes, the final lead to the tissue being some of the fluid in which the roots had been growing. As to the density of current used and the time of exposure, the author states: "A field of 5–20 volts per cm. was established for from 1 to 10 minutes, when the root was instantly fixed in acetic-absolute. Strength of field to which the living matter was actually exposed cannot be calculated." The effect produced was uniform, and varied only with intensity of current and time of exposure. The nucleus was usually slightly drawn out from a sphere to an ellipsoid, with the long axis parallel to the direction of the current flow. The nucleus maintained its position in the middle of the cell. The cytoplasm collected usually at the end of the cell toward the cathode, although frequently condensed into an equatorial plate. Within the nucleus the bulk of the solids collected at the side toward the anode. The nucleolus usually migrated toward the anode. No influence was exerted on division figures, spindles and chromosomes showing no sign of orientation or displacement whatever.

A careful review of the literature of this type of work reveals that current intensity was seldom measured accurately, and in most cases even when measured the results are not always reproducible because the organisms or organs studied were usually mounted in water, which acts as a partial conductor of current (conductivity varying with quantity of liquid used, electrolytes present, temperature, etc.), and in such experiments it is impossible to determine what part of the current flowed through the plant and what part through the water.

EFFECT OF CURRENT ON PARTICLES SUSPENDED IN LIQUIDS

The fact has long been known that finely divided particles suspended in water or other poorly conducting media will migrate, if the electric current is passed through the liquid, toward one or the other of the electrodes. Suspensions in water of starch, particles of paper, earth, asbestos, finely divided gold and copper all

move toward the positive pole. The particles of methyl violet, magdala red, lead, and bismuth move toward the negative pole.

REUSS (**20**) of Moscow seems to have been the first to discover the phenomenon of electrical migration variously known as electrophoresis or cataphoresis. He found that when two poles of a battery are immersed in a liquid and separated by a membrane the liquid will move through the membrane toward one of the two electrodes, and consequently the levels on the two sides of the membrane will not be the same. He discovered furthermore that while water moved toward the cathode, particles of various substances suspended in the water moved toward the anode.

That not all liquids migrate toward the cathode was first announced by QUINCKE (**19**). Oil of turpentine and absolute ethyl alcohol "that contained an organic impurity" migrated toward the anode. That the nature of the containers plays a part in determining the direction of flow was shown by the fact that in a glass tube lined with sulphur, oil of turpentine changed about in its direction of flow and migrated toward the negative pole. Water, however, was uninfluenced by the sulphur-lined tube and migrated, as in glass, toward the negative pole. The inhibiting influence of electrolytes on the movement of particles in suspension in an electric field was discovered by JÜRGENSEN (**11**). Suspensions of carmine in solutions of sulphuric acid, copper sulphate, and sodium chloride gave no evidence of movement when subjected to the current. On dilution of the easily conducting solutions, the particles again responded to the current.

That water, when absorbed by a semisolid material, will migrate was shown by DU BOIS-REYMOND (**7**). Incidental to this work was the invention of the non-polarizable electrode, which consists essentially of a short glass tube plugged at one end by moist kaolin, or by a camel's hair brush. Above the kaolin the tube is filled with a solution of $ZnSO_4$ into which dips an amalgamated zinc rod which is connected with the source of current. The semisolid used by DU BOIS-REYMOND was a cylinder of egg albumin. The non-polarizable electrodes were brought in contact with the ends of the cylinder, and in passing the current the end in contact with the positive electrode developed a constriction a short distance from

the surface of contact with the clay. The constriction became hard to the touch, the remainder of the cylinder swelling somewhat. When the current was reversed, the constricted end became soft and enlarged and the opposite end became constricted.

In repeating a part of JÜRGENSEN'S work, QUINCKE discovered that not under all conditions do the particles in suspension move toward the positive pole. Starch grains in water in a glass tube, as well as particles of silk, cotton, and paper migrated toward the positive pole when suspended in water, and toward the negative pole when suspended in oil of turpentine. The theory in explanation of these phenomena of poorly conducting liquids migrating in one direction and suspended particles in the opposite direction under the influence of the electric current, was propounded by HELMHOLTZ (10). The fundamental assumption of this theory is that at the surface of contact of any suspended particle there exists a double electric layer. If the particle bears a negative charge, the layer of medium immediately surrounding it bears a positive charge. On passing the current a displacement of one system against the other takes place, the liquid particles migrating toward one pole, the suspended particles toward the opposite pole. How the charge originates is not explained.

An explanation of the movement in opposite directions of oil of turpentine and water, and substances suspended in them, was first suggested by COEHN (5). He showed that with reference to the sign of the charge of a solid in contact with a liquid, the substance with the greater dielectric constant is positive to the other substance. The dielectric constant of oil of turpentine is 2.23, that of glass 4–7 (according to composition), and that of water is 81. In agreement with this explanation, glass is positive in oil of turpentine and negative in water. Water has a much higher dielectric constant than most other substances, and, as we have seen, most substances are negatively charged in water.

Observations on electrophoresis in colloidal "solutions" or sols were published by PICTON and LINDER (18) in 1892. Such colloidal sols consist essentially of very finely divided ultra-microscopic particles suspended in a liquid. Since LINDER and PICTON'S publication the work has been much extended, and the conclusions seem

inevitable that each colloidal particle bears a surface charge, which in some cases is negative and others positive, as indicated by the characteristic differences in the migration of different colloids. Furthermore, the researches of LINDER and PICTON show that the sign of charge possessed by the particles in a hydrosol bears a definite relation to the chemical composition of the particles, acid particles bearing a positive charge and basic particles a negative charge.

PERRIN (17) extended the work and formulated the following rule: "In the absence of polyvalent radicals, all non-metallic substances become positive in liquids that are acidic and negative in liquids which are basic."

HARDY (8) found that colloidal particles of derived albumins "move with the negative" (that is, they bear a negative charge and move toward the positive electrode) "if the reaction of the fluid is alkaline, with the positive stream if the reaction is acid."

The present work was undertaken with the idea of determining in a more or less quantitative way the effect of the direct electric current on the protoplast, the actual amount of current flowing through the organ being under control at all times. The investigation consists of two phases: first, that of cytological effect produced; and second, the combining of two factors, time and current intensity, to produce death of the protoplasts.

Materials and methods

Young seedlings of *Pisum sativum* of the variety known as White Canada Field Pea were chosen as the best material for this work, although onion, lupine, and Scarlet Runner bean were tested and the same cellular phenomena produced. The pea seedlings were grown in moist sawdust in ordinary 8-inch flower pots. The seeds were soaked in water for 12–24 hours, when all those not of uniform size were discarded. The hulls were removed and the seeds planted in the moist sawdust with the radicles pointing downward. The pots were then placed in the greenhouse. This method, if the moisture conditions of the sawdust are correct, will produce uniform germination and seedlings with straight radicles. Two lots were planted each day, one in the morning and one in the

evening, giving a choice of between 200–400 seedlings per day. The pots of seedlings were carried to the laboratory where the experimental work was done. In subjecting the seedlings to the current the following method was employed. The seedlings were subjected to the current one at a time in a moist chamber (text fig. 1) made of plaster of Paris and consisting of a box 18 cm. high, 10 cm. wide, and 7 cm. deep with one side open. A slab of plaster of Paris, cast to fit closely, was set against the open side to serve as a door. Through the top of this chamber the two non-polarizable electrodes were inserted. These were made as described earlier, but in actual practice it was also found that inserting the copper conducting wires without the zinc and zinc sulphate gave no polarization within 30 minutes, and with frequent changes of the moist kaolin was regarded as entirely safe.

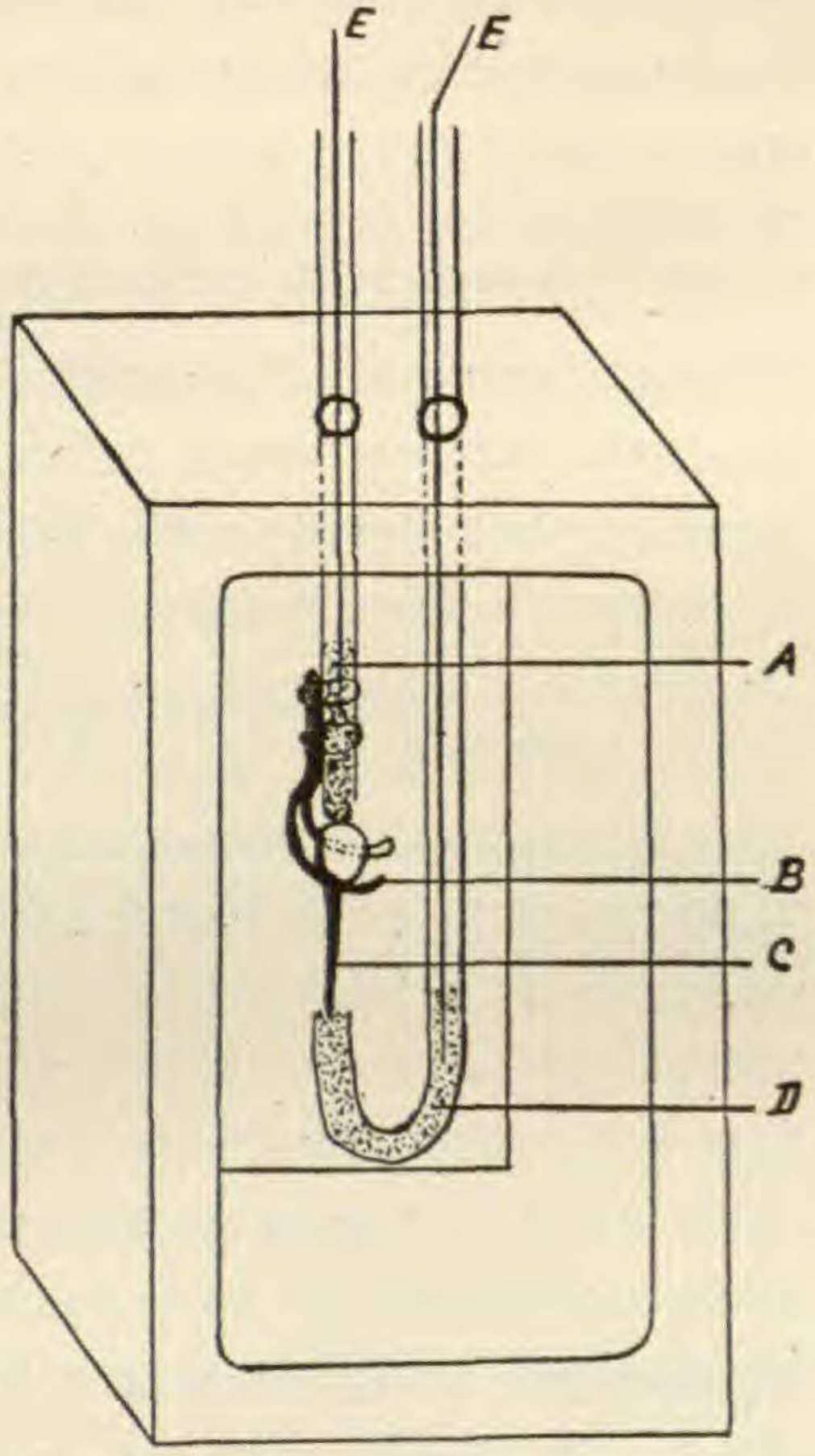

FIG. 1.—Moist chamber for exposing roots to current: E, E_1, wires leading to moist kaolin through glass tubes; A, D, kaolin in contact with extremes of seedling; C, root of seedling; B, glass fork for holding seedlings in position, and attached to glass tube of upper electrode by rubber bands.

Before setting up the electrodes, the moist chamber was placed in water for 20–30 minutes. This insured against the roots being exposed to a drying atmosphere while being exposed to the current. The arrangement of the conducting wires, together with resistances and measuring instruments, are diagrammed in text fig. 2. B and B^1, represented as binding posts in the diagram, consisted in reality of an ordinary lighting socket with key. From this socket a cord connected to an ordinary wall socket served as a source of current from the 110–120 volt direct current circuit. In some cases for added resistance a series plug with 4, 8, or 16 candle

power lamps was introduced at the wall socket. The current source in all cases, however, was the same. The sliding contact rheostat *R* has a resistance of 1770 ohms and a capacity of 0.45 amperes; while R^1 has a resistance of 464 ohms and a capacity of 1.2 amperes. The voltmeter *V* was attached to the binding posts *C*, C^1. The millivolt meter *A* (text fig. 2) was of Weston Electrical Instrument Company manufacture, with an upper range of 150 milliamperes and a lower range of 1.5 milliamperes. *M* represents the moist chamber.

The seedlings were carefully lifted from the sawdust, the adhering particles brushed off with a small camel's hair brush, the length measured with a small millimeter rule, and then by means of tweezers they were placed on the small glass fork which served as a

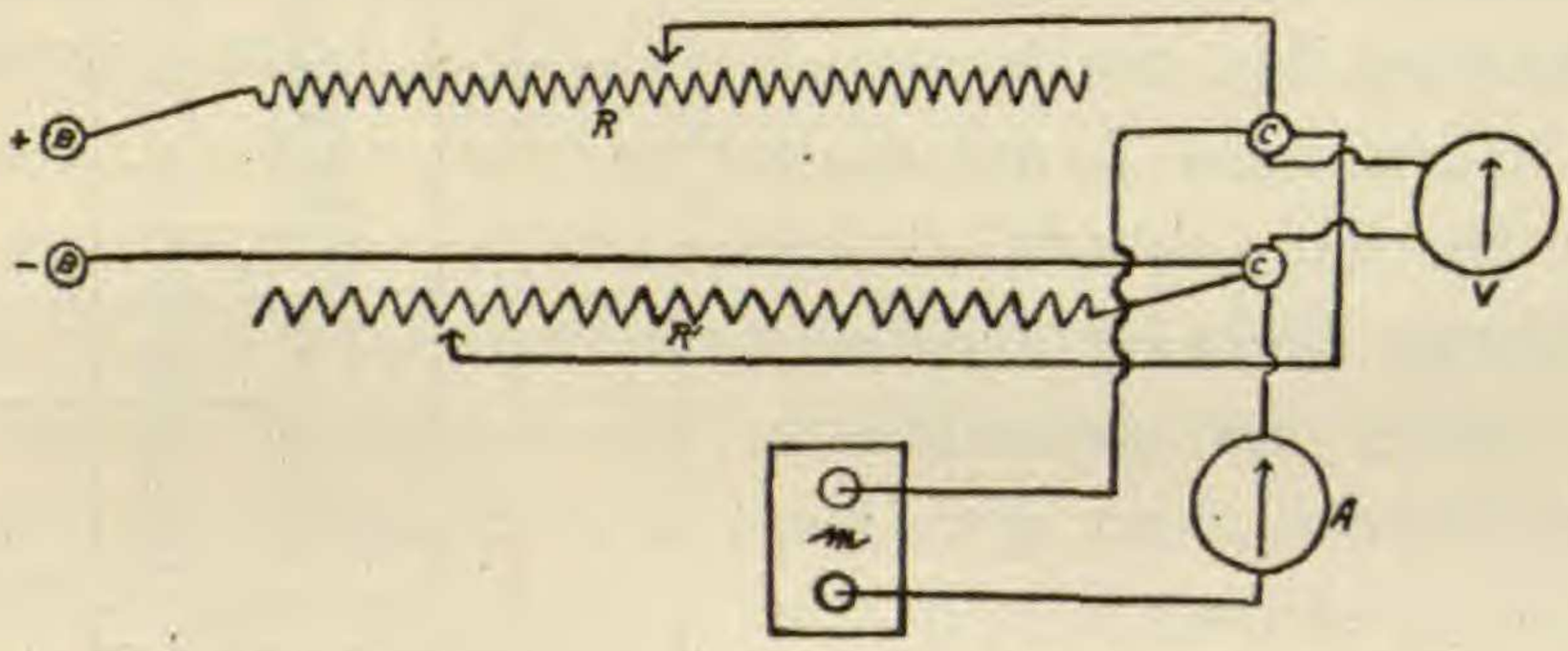

FIG. 2.—Apparatus and measuring instruments

support and which was attached to the upper electrode by means of a rubber band. The cotyledons of the seedling were now brought in good contact with the moist kaolin of the upper electrode, and the lower electrode (shaped like the letter J) moved upward so that the kaolin came in contact with not more than the first millimeter of root tip. The door was now placed in position and the current turned on with the key of the socket at *B*, B^1. The time of exposure was measured with a stop watch. During exposure voltage and amperage were read on the instruments.

For the cytological phase of the investigation the roots, immediately after exposure, were killed in Flemming's fluid. It should be stated that control seedlings were suspended in the moist chamber, in every case, near those being exposed to the current, and the roots killed and sectioned as the treated roots for comparison.

For that phase of the investigation relating to current intensity and time of exposure required to produce death, the seedlings were placed immediately after treatment in moist pine sawdust in glass battery jars. A stick the size of a lead pencil was pushed down next to the glass, the seedling placed in the opening thus made, and the sawdust carefully brought around the root. Figures on labels placed above each seedling served for identification when observation was again made, usually 24 hours after treatment. From 4–6 controls were placed in each jar of 12–15 treated seedlings.

All seedlings were carefully selected with special reference to length and diameter, those not corresponding to type being rejected. Only roots of 15–20 mm. length were used and of diameter as nearly uniform as examination without actual measurement could select. No practical means suggesting itself of measuring the diameter of the roots before exposure to current, the approximate diameter of the roots used was found as follows. Free-hand sections were made of 125 roots (carefully selected as if for treatment with the current), 3–4 mm. from the tip, the sections placed in a drop of water on a slide and diameter measured under the microscope with eyepiece micrometer. It was found that all fell within the following measurements: long diameter, 1.03–1.18 mm; short diameter, 0.84–0.96 mm. It will be seen from these dimensions that the roots were somewhat flattened. The cross-sectional area of the roots varied then from approximately 0.7 sq. mm. to 0.9 sq. mm. The diameter is an important factor, since on it depends the density of any given current intensity per unit area. It should be understood that these ranges are the extremes, the majority exhibiting no such variation as the extremes might indicate.

With the method described the actual current flowing through the tissue can be measured and read from the milliammeter, and calculated for the unit area. More or less just criticism is often made of the method of exposure to current of unicellular organisms in electrotactic experiments when the material to be examined is placed in liquid. The amount of current flowing through the organism depends therefore on whether or not the liquid surrounding it is a good conductor. If the liquid medium is a poor conductor, the current will pass in large measure through the organism;

if the opposite be true, very little if any of the current will pass through the organism. It is very evident that, since the conducting power of the medium is vastly increased by small amounts of electrolytes, the conductivity of the liquid is an ever-changing value. It is likewise evident that the same objection applies to roots in water through which the current is passing. Furthermore, the literature of electro-physiology is filled with references to current strength as weak, medium, strong, or with the mere statement of the number of cells used, with no statement of resistance in the circuit, so that actual current intensity cannot be determined, and difficulty is experienced in even approximating the conditions of the experiment. With the materials and methods just described these difficulties are avoided.

Observations

Killing effect of current

If a seedling with a root of 15–20 mm. in length and of 0.7–0.9 sq. mm. in cross-section is exposed in the manner described to a current of 0.3 milliampere, the following changes take place. In about thirty seconds the root begins to lose its normal color and becomes watery in appearance. If the current is continued for two minutes or longer, numerous very fine droplets of liquid appear over the surface 3–6 mm. from the tip. If the current is now stopped and the root tip tested, it will be found to be quite flaccid. Furthermore, measurement shows that the root is now from 0.5–1.0 mm. shorter than before the current was passed. An exposure of a longer period than two or three minutes results in the root becoming more or less translucent, and on testing after such longer exposure it is found to have become even more flaccid. In the preliminary experiments roots were treated with varying amperage and for different time periods, then fixed, sectioned, and stained. While the cytological pictures were similar except as to degree of intensity of results, they were not comparable with each other; for example, it was difficult to produce the same result with a 0.4 milliampere current and a 0.5 milliampere current by varying the time.

After much experimentation, in an effort to arrive at somewhat comparable cytological results, had ended in failure, it was found

possible to establish in a fairly definite way the quantity of current and the time required to produce death of the root. This was accomplished by numerous trials using a constant amperage, and varying the time factor until exactly the time exposure required (using that particular amperage) to produce death was determined. This method yielded comparable cytological results. Current and time factors were varied from 0.6 milliampere for fifty-two seconds to 0.05 milliampere for thirty to thirty-five minutes. Longer exposures were also made for as long as two hours at 0.01 milliampere.

In the determination of whether or not a certain exposure to current produced death, roots immediately after treatment were planted in moist sawdust as previously described. Examination was made after a lapse of twenty-four hours and record of condition made. Roots so treated, current intensity of 0.3 milliampere for two and a half minutes or longer, will after twenty-four hours appear a chalky white at extreme tip and exhibit considerable shriveling in the region of rapid elongation. If such a root is tested by being drawn lightly between thumb and forefinger, it offers little resistance, and flattens readily. It is quite evident that the entire root tip of 1.5 cm. is dead. If subjected for a less period than two and a half minutes to this current intensity, a majority of the roots will show the shriveling in the region of rapid elongation and above, but the first 4–5 mm. of the root will be more or less translucent, quite different in appearance from the chalky white previously described, and quite firm to the touch. It is evident in these cases that such roots are dead above the first 4–5 mm. of the tip, and that the cells are still in a living condition in the extreme tip. This conclusion is further strengthened by the fact that in a great many cases these roots will show curvatures at the tip. These curvatures take no specific direction with reference to how the current is applied. The seedlings were always set into the apparatus with the cotyledons toward the front, yet the curvatures appeared in every plane, and varied from a slight crook to a right angle curve, or in some cases even a bending back on the main axis to a U-shape. These curvatures suggest unilateral injury which stained preparations in no case reveal, and indicate a problem of great interest upon which it is planned to do further work.

The criteria by which a root was judged to be dead, living, or partly dead are the following. When a root after twenty-four hours was distinctly shriveled and drying in the upper region, that

TABLE I

CURRENT 0.6 MILLIAMPERE, 120 VOLTS; CRITICAL TIME 52 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1.....	5	↑	15	+0.5	4	5	0
2.....	5	↑	30	+0.2	3	5	0
3.....	5	↑	40	−0.4	4	4	1
4.....	5	↓	45	−0.5	3	5	0
6.....	5	↑	50	−0.4	3	3	2
9.....	6	↓	50	−1.0	2	2	4
11+15.....	10	↑	52	−1.1	0	0	10
13+15.....	10	↓	52	−1.2	1	1	9
7+14.....	10	↓	55	−1.3	0	0	10
10+12.....	11	↑	55	−1.1	1	1	10
5.....	5	↑	60	−0.9	0	0	5
8.....	5	↑	60	−0.16	0	0	5

TABLE II

CURRENT 0.5 MILLIAMPERE, 110 VOLTS; CRITICAL TIME 65 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1.....	5	↑	20	+1.0	3	5	0
2.....	5	↑	40	+0.1	3	4	1
4.....	5	↓	45	−0.2	4	4	1
5.....	5	↓	50	0.0	4	4	1
6.....	5	↓	55	−1.1	2	2	3
8.....	5	↑	55	−0.1	3	4	1
7+13.....	10	↓	60	−0.3	1	2	8
3, 9, 11.....	15	↑	60	−0.6	5	4	11
15, 17, 19.....	16	↑	60	−0.7	3	6	10
10+16.....	12	↓	65	−0.7	1	1	11
14+18.....	10	↑	65	−1.1	0	0	10
12.....	5	↑	70	−0.8	0	0	5
20.....	5	↓	70	−1.0	0	0	5

portion was regarded as dead. In not a single case in all tests made did this part revive after having reached this stage. A chalky white appearance of the first 4 or 5 mm. of tip, together with the

exhibition of loss of turgidity, by showing little resistance to flattening by gentle pressure (being drawn between thumb and forefinger) was regarded as evidence that this portion of the root was

TABLE III

CURRENT 0.4 MILLIAMPERE, 100 VOLTS; CRITICAL TIME 90 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	6	↑	30	+1.1	4	6	0
2	6	↑	35	+2.0	5	6	0
3	5	↑	40	+1.4	3	5	0
4	8	↑	45	+0.8	5	8	0
5	6	↓	50	+1.5	3	6	0
6	6	↑	55	−0.1	3	5	1
7	6	↓	60	−0.5	2	4	2
8+10	12	↓	65	−0.5	7	9	3
9+11	12	↑	70	−0.5	6	8	4
14	6	↓	70	−0.3	5	5	1
12	6	↑	75	−0.5	4	6	0
13+15	13	↑	80	−0.0	5	8	5
23+25	10	↑	85	−0.6	2	3	7
18+27	10	↓	90	−1.0	0	0	10
16, 24, 26	21	↑	90	−0.7	2	2	19

TABLE IV

CURRENT 0.3 MILLIAMPERE, 90 VOLTS; CRITICAL TIME 2 MINUTES, 30 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	6	↑	50	+1.5	3	6	0
2	6	↓	120	−0.5	2	4	2
3	6	↑	120	−0.3	4	5	1
4+9	16	↑	135	−0.5	0	6	10
6	7	↓	135	−0.7	2	3	4
12	5	↑	140	−0.6	1	1	4
5, 7, 13	28	↑	150	−0.7	0	0	28
10	10	↓	150	−1.0	0	0	10
8+11	18	↑	165	−0.9	0	0	18

dead. When, however, the appearance of the extreme tip was watery instead of chalky white and exhibited distinct turgidity, it was regarded as evidence that the cells were in a living condition. Associated with the latter condition was an increase in length and

the curvatures mentioned. It is entirely possible for the upper portion of the root (the region of rapid elongation and above) to be dead and the tip to continue growth for a short time; in fact, many such cases were found. It is well understood that it is difficult to

TABLE V

CURRENT 0.2 MILLIAMPERE, 70 VOLTS; CRITICAL TIME 4 MINUTES

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
11	5	↓	220	−0.1	1	5	0
3+7	10	↑	225	−0.6	0	4	6
9+12	10	↑	230	−0.6	2	4	6
1+8	21	↓	240	−0.4	0	3	18
4+10	15	↑	240	−0.7	0	0	15
2	8	↑	270	−0.9	0	0	8
5	5	↓	270	−0.4	0	0	5
6	5	↑	300	−0.6	0	0	5

TABLE VI

CURRENT 0.15 MILLIAMPERE, 50 VOLTS; CRITICAL TIME 6 MINUTES, 15 SECONDS

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1+12	10	↑	300	−0.4	1	7	3
2, 13, 16	15	↓	360	−0.8	4	6	9
4, 8, 18	16	↑	360	−0.1	4	15	1
19+27	8	↑	370	−0.4	1	2	6
26	5	↓	375	−0.2	0	0	5
14, 17, 21	15	↑	375	−0.7	1	1	14
22, 25	10	↑	375	−0.7	0	0	10
5	5	↑	390	−0.5	0	0	5
10, 15, 23	15	↑	390	−0.5	0	0	15
3, 6, 7	15	↑	405	−0.5	0	0	15
9+11	11	↓	405	−0.6	0	0	11

set up a criterion of measurement as to when death takes place; in fact, death has been called by some physiologists "a reversible process." The writer believes, however, that these criteria are sufficiently definite as used, and that they are scientifically sound.

In determining the time factor, the current was kept at constant amperage by sliding resistances for a definite time period, the time being measured by a stop watch. The usual practice was to treat seedlings in succession in the same manner (as to time and current),

TABLE VII

CURRENT 0.1 MILLIAMPERE, 40 VOLTS; CRITICAL TIME 9 MINUTES

Lot number	Number of roots used	Direction of current	Time of exposure in seconds	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	5	↑	420	0.6	5	5	0
2+18	10	↓	480	−0.2	1	6	4
3, 5, 8	11	↑	480	−0.2	0	7	4
4, 21	8	↑	510	−0.7	0	1	7
23, 25	9	↑	525	−0.4	1	2	7
6, 9, 24	13	↓	550	−0.5	0	2	11
10, 14, 19, 22	20	↑	540	−0.4	0	1	19
16	5	↓	570	−0.7	0	0	5
15+17	9	↑	600	−0.6	0	0	9
26	10	↓	600	−0.7	0	0	10

TABLE VIII

CURRENT 0.05 MILLIAMPERE, 30 VOLTS; CRITICAL TIME PROBABLY BETWEEN 32 AND 35 MINUTES

Lot number	Number of roots used	Direction of current	Time of exposure in minutes	Average loss (−) or gain (+) in length in mm.	Number showing curvature	Dead above only	Dead above and at tip also
1	3	↑	15	−0.5	0	3	0
2	1	↑	18	−0.2	0	1	0
3+8	7	↑	25	−0.2	0	3	4
10	5	↓	28	−0.1	1	1	4
9, 11, 12	16	↑	30	−0.3	3	3	13
13	6	↑	32	−0.4	1	1	5
6	2	↑	35	−0.5	0	0	2
14	4	↓	35	−0.5	0	0	4
4	2	↓	40	−0.2	0	0	2
7	2	↑	40	−0.3	0	0	2
5	2	↓	50	−0.5	0	0	2

such group (usually five), being designated as a "lot" in the tables. Roots were exposed for a time, calculated from preliminary experiments to be below that required to kill, then time of exposure increased for next lot and so on until all were killed. When ninety

per cent or over were killed at any particular combination of current intensity and time, this was regarded as the death point. Death points were determined for current intensities of 0.6 to 0.05 milliamperes as shown in the tables. Direction of flow of current is indicated by arrows. When the current was applied with the positive electrode at the tip, the arrow points upward. Under "average loss or gain" is given the loss or gain in length over measurement taken just previous to exposure. Final measurement was always taken twenty-four hours after exposure. Under the heading "dead above only" is given the number in which the region above the first 4 or 5 mm. was killed. This effect was often noticeable as far back

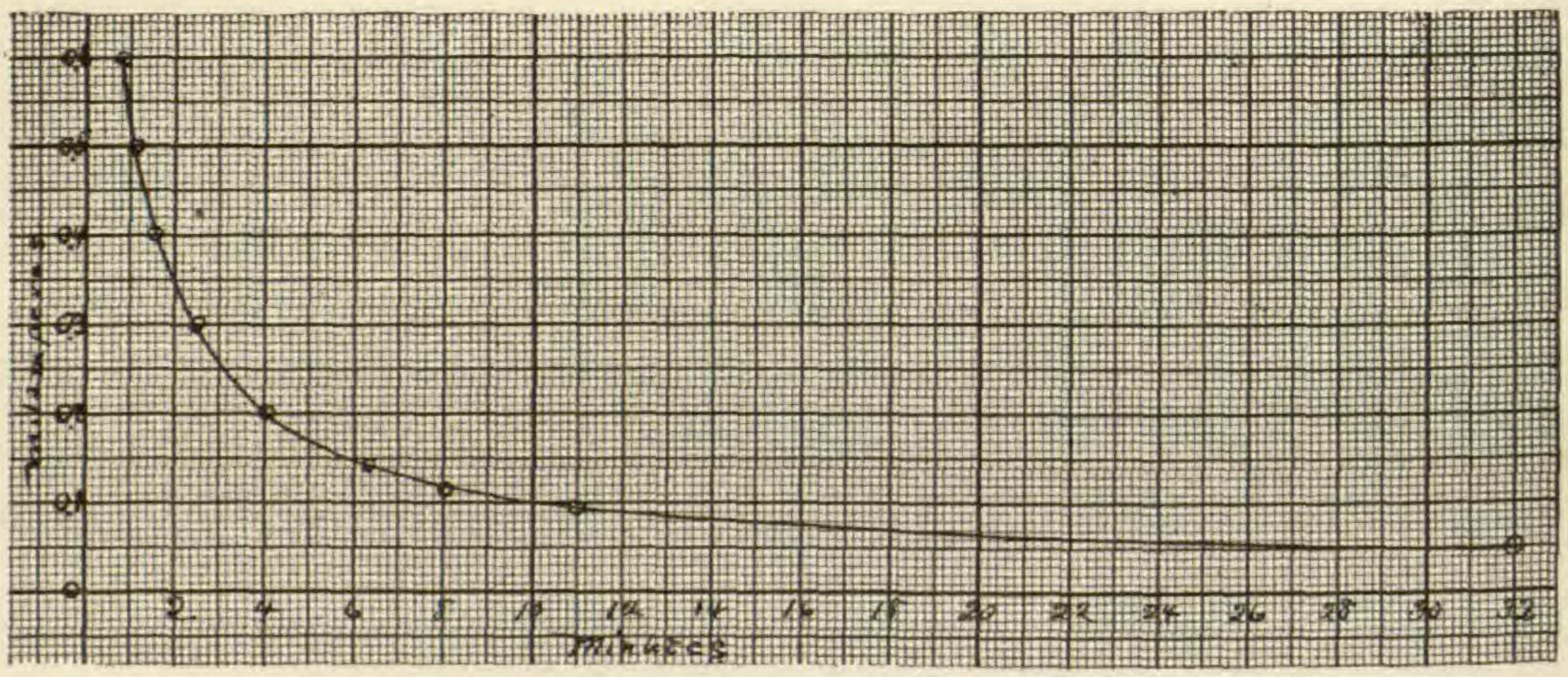

FIG. 3.—Current and amperage required to produce death in roots of 0.7–0.9 sq. mm. cross-sectional area.

as the cotyledons. The last column gives the number in which the entire root was killed. Thus it can be seen from the tables that the critical time at 0.6 milliampere is fifty-two seconds; at 0.5 milliampere sixty-five seconds; at 0.4 milliampere ninety seconds, etc. It should be stated that controls were planted with each lot, and that all showed a decided increase in length, the increase varying from 12–19 mm. at the end of twenty-four hours.

Plotting the points as shown in the tables into a graph gives the death curve (text fig. 3). With current of 0.05 milliampere the death point was determined with great difficulty, due to length of exposure required, which had a tendency to dry out the root. The critical time seems to lie between thirty and thirty-five minutes.

It is not contended that the death curve is sharp and definite. As the tables show, some roots were killed before the maximum was reached. This difference is undoubtedly accounted for by the variations in cross-sectional area of roots used.

Cytological

A rather definite cytological effect is produced in roots exposed to the current just long enough to produce death. Roots exposed for fifty-two seconds at 0.6 milliampere give a very similar picture to roots exposed for two and a half minutes at 0.3 milliampere, or any other point on the death curve. Similarly, roots exposed for any fraction of the time required to produce death at any amperage give a comparable picture with those produced by exposure for the same fraction of time required to produce death at any other amperage. This fact was established only after long experimentation, and until then no comparable cytological results could be obtained with various combinations of time and current. The direction of current through the root had no influence except in direction of migration.

Best results in fixing were obtained by various combinations of Flemming's fluid. Root tips immediately after treatment were placed in the killing fluid. The usual processes incident to the paraffin method were used, and the roots sectioned and stained in saffranin-gentian violet. Satisfactory staining was quite difficult to obtain, and the finer details of the treated protoplasts in most cases were difficult to distinguish.

In a root exposed to the current just long enough to produce death, the cells of the central cylinder back of the root cap show fairly even distribution of cytoplasm, which, however, is coarsely granular compared with the controls. Many cells show a distinct migration of cytoplasm toward the positive electrode. It is in the nuclei, however, that the effect is more noticeable. The nucleolus may have been displaced in either direction, more cells, however, showing displacement toward the positive electrode. A majority of the nucleoli had become elongated in a direction at right angles to the long axis of the root, similar to that shown in fig. 3. The nucleolus frequently is elongated sufficiently to reach

across the nuclear cavity. Other dense material within the nucleus is in every case deposited in a crescent-shaped mass against the nuclear membrane toward the positive electrode. This is shown in some of the cells of figs. 3 and 5. The chromatin appears very coarsely granular. Occasionally a cell is found in which a small amount of this granular material is deposited against the side of the nuclear membrane toward the negative electrode, leaving a clear central space across which lies the much flattened nucleolus.

The cells of the central cylinder about 1 mm. from the cap show shrinkage which is evident at the ends of the cells, but not laterally.

In the cortex of the first millimeter the cells show greater effect than in the central cylinder. The cytoplasm in these cells is in nearly every cell definitely aggregated against the wall toward the positive electrode. The nucleolus no longer lies across the nuclear cavity, but has migrated with the chromatin toward the positive electrode (fig. 8). The nuclear cavity is no longer spherical but egg-shaped, with the smaller end toward the anode. Chromatin and nucleolus are packed into the small end, and seem to have forced distention of the nuclear cavity. Frequently fine granular threads radiate from this mass toward various points in the periphery of the nucleus, as shown in figs. 8 and 11. This bears a striking resemblance to fig. 12, pl. 18, of MOTTIER's paper (**16**). The nucleolus in most cells at this stage cannot be distinguished as a separate body from the chromatin.

It is in dividing cells that the greatest effect of the current might perhaps be expected, yet such is not the case. Migration does not take place at all in a cell in the process of nuclear division in either cytoplasm or chromosomes. Staining of division figures is poor, and in most cases presents a blurred picture in which the chromatin has the appearance of having melted together. Cells with nuclear division are shown in figs. 2 and 3. This blurred condition is characteristic of all division figures, no matter at what stage. All parts of such a cell usually stain a deep red with saffranin. The absence of migration should be expected in such cells from results found by KITE (13) and CHAMBERS (4), who both found the protoplast during division in a very viscous state, in the form of a gel.

KITE states that when such a protoplast was cut into, the pieces retained their shape definitely and behaved distinctly as a gel.

In the region of rapid elongation in both cortex and central cylinder, 3–5 mm. from the cap, the cytological picture reveals little effect compared with the extreme tip. The cytoplasm shows no migration whatever, although it is more coarsely granular than that of the controls. The nucleolus retains its form and position, while the chromatin is aggregated in a crescent-shaped mass against the nuclear membrane toward the positive electrode in a majority of cells, in a few toward the negative electrode. In some cases, however, the entire nuclear material is displaced within the cavity (fig. 6), and in a few cases even the entire nucleus lies in a dense mass against the wall toward the positive electrode, a phenomenon not met with at this exposure in cells nearer the cap. Shrinkage is quite evident in the region of rapid elongation.

The root cap rarely shows any displacement of either cytoplasm or nucleus, doubtless largely due to the fact that the moist kaolin of the electrode is a better conductor than the root, and so very little current passes through the cap.

The first noticeable cytological effect of the current is produced on exposure of approximately one-tenth of the time required to produce death, and the first visible reactions occur in the cortical region about 1 mm. above the cap. Such cells show large vacuoles in the end toward the negative electrode, and the cytoplasm appears slightly more granular than in the controls. The chromatin at this stage is beginning to migrate toward the positive electrode. The nucleolus lies in its normal position, but soon after one-tenth of time exposure begins to show the flattening previously described. At this stage the region above the first millimeter shows nothing abnormal, neither do the cells immediately behind the central portion of the root cap. The effect of the current is progressive and the results are cumulative. At one-half of the time for death point, the nucleolus flattens and the cytoplasm definitely begins to migrate. With an exposure of three-fourths of the time for death the nucleolus and chromatin have migrated toward the positive electrode, the cytoplasm being very coarsely granular and exhibiting a greater amount of migration than in the previous

stage. The picture at death point has already been described in detail.

If the current is continued for a longer period than is necessary to produce death, all protoplasmic contents, especially the cortical cells of first 3–4 mm. of tip, are aggregated in a dense mass against the cell wall toward the positive electrode, as shown in pl. II, also partially shown in figs. 9 and 10.

Discussion

Within thirty seconds after the current is applied, the resistance in the circuit falls considerably. The resistance, however, was always kept constant by means of the sliding rheostats. Coincident with the drop in resistance, the tiny droplets of liquid appeared on the surface of the root in the region of rapid elongation and above. This suggests increased permeability of the protoplasts and of the cell walls to liquids of the cell sap, and further suggests increased freedom of movement of particles in the sap or cytoplasm or both. The consequent loss of turgidity and shortening of the root substantiate this view.

It is most interesting to find that the greatest visible effect of the current is not in the region of most rapidly dividing cells, but slightly farther from the tip. This suggests that the protoplasm of these cells exists in a much more viscous state than in cells farther from the tip. The lack or presence of free ions would influence conduction of current. It is possible that free ions exist in increasingly greater number with the absorption of water from the primordial meristem to the region of greatest elongation. If this assumption is true, we would expect least effect of current in the primordial meristem, where the cytoplasm would be viscous and behave as a gel, and a greater effect where the cytoplasm became more nearly semi-fluid, and least effect where the free ions of the cell sap conducted the current almost altogether. This assumption agrees with the facts, for the least (or no) migration occurs in the cells with large vacuoles.

In no preparations made could any basis be found for HARDY'S (9) statement that the cytoplasm migrates to the wall, loses its original charge, gains one of opposite sign, and then migrates toward

the opposite end of the cell. This statement, however, may apply to the chromatin. No theory is advanced as to why neighboring cells behave variously in this regard. The relative hydrogen ion concentration of the different parts of the cell no doubt plays a large part with reference to its reaction to the current. The assumption is generally made that cytoplasm is weakly alkaline. This conception is probably based on the reaction of the cell sap; nevertheless we have evidence that the migration of the protein constituents in the cells is toward the positive electrode. Likewise it is in accord with HARDY'S (9) results in treatment of a derived albumen, first with acid, then alkali, and a consequent change in direction of migration as previously stated. That the chromatin under some conditions bears a positive charge seems to be suggested by my own experimental evidence. That the migration of protoplasmic particles, as influenced by the current, is due to the particular electrical charge of the constituent colloidal particles also seems probable, and would suggest that the cytoplasm carries (in the roots of the plants used in this study) always a negative charge.

Summary

1. A method of subjecting roots in moist air to the direct electric current has been devised which makes it possible to control and accurately measure the current actually flowing through the root. There is no evidence that under such conditions roots behave differently from those in soil or liquid if subjected to the same current intensity.

2. Combinations of current intensity and time factors have been determined for producing death of the cells of roots, and the death curve plotted.

3. Cytological preparations of treated roots show a migration of cell contents (with few exceptions) toward the positive electrode.

4. The migratory effect (transfer of material) is not the same for all regions of the root. With one-tenth of time of death current, the cells immediately back of the root cap show little effect, those a little older (1 mm. back) greatest effect, and the cells with large vacuoles no or little effect as to cytoplasm.

5. It is suggested that, with addition of water, more free ions may occur to conduct the current; that the protoplasts of the primordial meristem are in a state of gel.

6. It is further suggested that the difference in true acidity, H-ion concentration of various protoplasts, may account for occasional different behavior of adjacent cells.

7. The theory of electrophoresis probably accounts for the migration phenomena, assuming that the constituent colloidal particles of protoplasm bear an electric charge.

8. Assuming an electric charge carried by such particles, it would follow that the particles of the cytoplasm of the cells of the roots of the Canada Field Pea bear a negative charge, and that the chromatin particles, in some cases only, may bear a positive charge.

The writer takes pleasure in extending thanks to Dr. W. G. MARQUETTE and Professor R. A. HARPER for kindly suggestions and criticism freely given throughout the progress of the work. The work was done in the laboratories of the Department of Botany, Columbia University, New York City.

SYRACUSE UNIVERSITY
SYRACUSE, N.Y.

LITERATURE CITED

1. AMICI, G. B., Osservazioni sulla Circulazione del Succhionella *Chara*. Mem. Matem. Soc. Ital. VIII. **2**: Med. 1818 (quoted from BECQUEREL).
2. BECQUEREL, A. C., Influence de l'électricité sur la circulation du *Chara*. Compt. Rend. **5**:784–788. 1837.
3. CARLGREN, O., Über die Einwirkung des constanten galvanischen Stromes auf niedere Organismen. Archiv Anat. und Physiol. 1900 (pp. 49–75).
4. CHAMBERS, R., Changes in protoplasmic consistency and their relation to cell division. Jour. Gen. Physiol. **2**:49–68. 1919.
5. COEHN, A., Über ein Gesetz der Electricitätserregung. Ann. Physik und Chemie **64**:217–232. 1898.
6. DALE, H. H., Galvanotaxis and chemotaxis of ciliate infusoria. Jour. Physiol. **26**:291–361. 1911.
7. DU BOIS-REYMOND, E., Über die innere Polarisation poröser mit Elektrolyten getränkter Halbleiter. Berlin Akad. Wiss. 1856 (pp. 450–468) and 1860 (pp. 846–906).

8. HARDY, W. B., The coagulation of proteid by electricity. Jour. Physiol. **24**:288–304. 1899.
9. ———, Note on difference in electrical potential within the living cell. Jour. Physiol. **47**:108–111. 1913.
10. HELMHOLTZ, H. VON, Studien über elektrische Grenzschichten. WIEDEMANN'S Annalen **7**:337. 1879; also Mem. Physical Soc. London **1**:1–42. 1888.
11. JÜRGENSEN, T., Über die Bewegung fester, in Flüssigkeiten suspendierter Körper unter dem Einfluss des elektrischen Stromes. REICHERT und DU BOIS-REYMOND Archiv. Anat. und Physiol. 1860 (pp. 673–687).
12. ———, Über die in den Zellen der *Vallisneria spiralis* stattfindenden Bewegungserscheinungen. Studien Physiol. Inst. Breslau 1861 (pp. 87–109).
13. KITE, G. L., Studies in the physical properties of protoplasm. Amer. Jour. Physiol. **32**:146–164. 1913.
14. KÜHNE, W., Untersuchungen über das Protoplasma und die Contractilität. 1864.
15. LILLIE, R. S., On difference in the direction of the electrical convection of certain free cells and nuclei. Amer. Jour. Physiol. **8**:273–283. 1903.
16. MOTTIER, D. M., The effect of centrifugal force upon the cell. Ann. Botany **13**:325–360. 1899.
17. PERRIN, J. (Électricité)—"Examen des conditions qui déterminent le signe et la grandeur de l'osmose électrique et de l'électrisation par contact." (Note de M. JEAN PERRIN, présentée par M. MASCART.) Compt. Rend. **136**:1388–1391. 1903.
18. PICTON, H., and LINDER, S. E., Solution and pseudo-solution. Jour. Chem. Soc. **61**:148. 1892; **67**:63. 1895; **71**:568. 1897; **87**:1906. 1905.
19. QUINCKE, G., Über die Fortführung materieller Theilchen durchströmender Elektricität. Pogg. Annalen IV. **23**:513–598. 1861.
20. REUSS, F. F., Mein. Soc. Imp. Nat. Moscou **2**:327. 1809 (quoted by JÜRGENSEN).
21. VERWORN, M., Die polare Erregung der Protisten durch den galvanischen Strom. PFLUGER'S Archiv. **5**:1–36. 1889; **46**:267–303. 1890; **62**:415–450. 1896; **65**:47–62. 1897.
22. WIEDEMANN, G., Über die Bewegung im Kreise der geschlossenen galvanischen Säule. Pogg. Annalen **87**:321–352. 1852.

EXPLANATION OF PLATES II, III

PLATE II

Longitudinal sections of root tips: magnification shown by scale; smallest spaces equal to 0.01 mm.; ×110.

A.—Root exposed to current of 0.4 milliampere for one and a half minutes; positive electrode at tip.

B.—Root exposed to current of 0.2 milliampere for four minutes; negative electrode at tip.

C.—Control, not exposed to current.

PLATE III

Cells from various portions of roots exposed to current, and from controls; magnifications for all figures approximately 580 diameters, with exception of fig. 8, about 500 diameters, and fig. 10, 550 diameters; all photomicrographs mounted so as to have positive electrode below.

FIG. 1.—Normal cells (not exposed to current) 8–10 mm. from tip in cortex.

FIG. 2.—Cells about 1 mm. from tip in central cylinder from root exposed to current of 0.5 milliampere for sixty-five seconds; in the lower right hand protoplast dividing.

FIG. 3.—Cells from cortex of root exposed to current of 0.2 milliampere for one minute, this being one-fourth time required to kill root at this amperage.

FIG. 4.—Normal cells from cortex about 3 mm. from tip.

FIG. 5.—Cells of central cylinder about 8 mm. from tip; root exposed to current of 0.6 milliampere for twenty-five seconds.

FIG. 6.—Cells from cortex about 10 mm. from tip; root exposed to very severe treatment (ten minutes at 0.4 milliampere).

FIG. 7.—Normal cells from cortex about 1 mm. from tip.

FIG. 8.—Cells from cortex about 1 mm. from tip of root exposed to current of 0.6 milliampere for forty seconds.

FIG. 9.—Cells from cortex about 4–5 mm. from tip; cytoplasm and nuclei also displaced; current of 0.3 milliampere for two and one-half minutes.

FIG. 10.—Same as fig. 9 but exposed to current only one and one-half minutes.

FIG. 11.—From same region as fig. 8